Photosensitizers for the Accelerated Degradation of Chlorinated Cyclodienes and Other Insecticide Chemicals Exposed to Sunlight on Bean Leaves

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Rotenone and some related substituted-4-chromanones act as photosensitizers to accelerate the photoalteration of chlorinated cyclodiene insecticide chemicals exposed to sunlight on bean leaves. The rate of dieldrin photoalteration is also increased, although to a lesser extent, by certain triplet-state photosensitizers. In addition, rotenone and the chlorophylls from spinach chloroplasts sensitize the photodegradation of several other types of insecticide chemicals, including organophosphate compounds, methylcarbamates, pyrethroids, and dinitrophenol derivatives. Further studies on pesticide chemical–photosensitizer interactions may reveal combinations useful in manipulating the persistence of pesticide chemical residues on treated crops and in the environment.

Sunlight is effective in decomposing many pesticide chemicals when they are exposed on silica gel chromatoplates in the presence of appropriate photosensitizers (Ivie and Casida, 1971). Certain chlorinated cyclodiene insecticides are particularly susceptible to photoalteration when sensitized by selected compounds of high triplet-energy state and by a few commonly used pesticide chemicals (Ivie and Casida, 1970, 1971).

The photochemical isomerization reactions known for certain chlorinated cyclodienes are shown in Figure 1. A dieldrin isomer, photodieldrin (Robinson et al., 1966; Rosen et al., 1966), occurs to a small extent on some foodstuffs (Robinson et al., 1966) and in soils treated with abnormally high aldrin levels (Lichtenstein et al., 1970). Aldrin forms photoaldrin by an analogous isomerization reaction (Rosen and Sutherland, 1967), whereas isodrin and heptachlor are converted to doubly-bridged, birdcage isomers on exposure to ultraviolet light in the presence of the sensitizer benzophenone (Rosen et al., 1969). Endrin is converted to the ketone and aldehyde isomers both thermally (Phillips et al., 1962) and by ultraviolet light (Rosen et al., 1966). With the exception of photodieldrin, the cyclodiene photoisomers have not been reported as environmental contaminants.

The toxicity to insects and mammals of the photoisomers relative to the parent compound varies with the cyclodiene involved. While photoisodrin is less toxic than isodrin (Khan et al., 1970; Rosen et al., 1969) and the ketone and aldehyde isomers of endrin are much less toxic than endrin (Phillips et al., 1962; Rosen et al., 1966), photodieldrin, photoaldrin, and photoheptachlor are at least as toxic as their respective parent compounds (Khan et al., 1970; Rosen and Sutherland, 1967; Rosen et al., 1966, 1969). Thus, it is important to consider the extent of photoisomerization in evaluating the toxicity and significance of environmental residues of these insecticide chemicals.

The present investigation and a preliminary report on this study (Ivie and Casida, 1970) consider the interactions of photosensitizers with pesticide chemicals and particularly chlorinated cyclodienes exposed to sunlight on plant foliage. In addition, the possibility that there are natural photosensitizers within plant tissue that affect the persistence of some pesticide chemicals is evaluated in studies involving the use of aqueous suspensions of chloroplasts.

MATERIALS AND METHODS

Chemicals. Structures for the pesticide chemicals or their derivatives or analogs designated here by common or trade names are given by Crombie (1963), Kenaga and Allison (1969), Martin (1968), or in the Merck Index (1968).

Sources for the unlabeled chemicals were as follows: J. D. Rosen (Rutgers University, New Brunswick, N.J.) for the photoisomers of aldrin, dieldrin, heptachlor, and isodrin; S. B. Soloway (Shell Development Co., Modesto, Calif.) for the isomers and analogs of endrin; pesticide chemicals from the basic manufacturers; photosensitizers and analogs from the Photosensitizer and Quencher Kit (Commodity No. Z901, J. T. Baker Chemical Co., Phillipsburg, N.J.) or from chemical supply houses; rotenoids and related compounds from Leslie Crombie (University College of South Wales and Monmouthshire, Cardiff, U.K.), Masanao Matsui (University of Tokyo, Japan), and Minoru Nakajima (University of Kyoto, Japan).

The C^{14}-labeled pesticide chemicals studied included the

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Photoaldrin
formation

Photodieldrin
formation

Photoisodrin
formation

Photoheptachlor
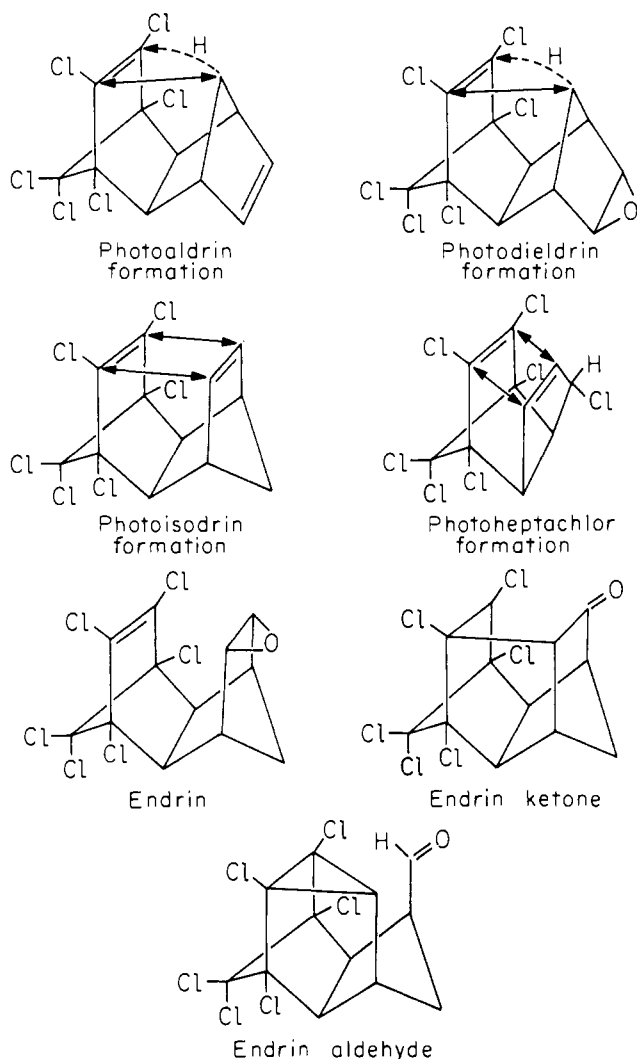formation

Endrin

Endrin ketone

Endrin aldehyde

Figure 1. Structures of aldrin, dieldrin, isodrin, and heptachlor, indicating the reactions involved in photoisomer formation, and of endrin and its two photoisomers, endrin ketone and endrin aldehyde. The cyclization reactions involve new bond formation (↔), resulting in saturation of the original double bond(s), accompanied in aldrin and dieldrin by hydrogen migration (⇢). Epoxide ring opening with new bond formation, hydrogen migration, or carbon–carbon bond cleavage results in the formation of the ketone and aldehyde isomers of endrin

compounds described previously (Ivie and Casida, 1971). The specific activities were adjusted to 0.5 to 1.5 mCi/mmol by dilution with unlabeled materials.

Chromatography. Thin-layer chromatography (tlc) of radio-labeled compounds was accomplished with silica gel F_{254} precoated chromatoplates (gel thickness of 0.25 mm, 20 × 20 cm glass plates, Merck AG, Darmstadt, Germany). After development of the chromatoplate in an appropriate solvent system, radioactive products were detected by radio-autography and quantitated by scraping the radioactive gel regions from the plate and counting in a Beckman LS-150 liquid scintillation spectrophotometer equipped with external standardization for quench correction. The labeled compounds studied and the tlc solvent systems used were: aldrin (hexane-benzene, 4:1); dieldrin and endrin (hexane-ethyl acetate, 4:1); DDT (hexane-benzene, 5:2); diazinon, Dyfonate, Imidan, and Sumithion (hexane-acetone, 4:1); malathion (hexane-acetone, 3:2); Baygon (chloroform-n-butanol-ether, 100:4:1); carbaryl (ether-hexane, 2:1); isolan (ethyl

acetate-ethanol, 49:1); Mesurol, pirimicarb, and Zectran (ether-hexane-ethanol, 77:20:3); SD-8530 (ether-hexane, 4:1); allethrin and pyrethrin I (benzene saturated with formic acid-ether, 10:3); rotenone (benzene-methanol, 9:1); Dessin and DNBP (hexane-benzene-ether, 4:1:1); piperonyl butoxide (benzene-methanol, 5:1); and 2,4-D (benzene saturated with formic acid-ether, 2:1). In studies with unlabeled chlorinated hydrocarbons, the tlc plates were prepared by slurrying aluminum oxide in ethanol containing sufficient silver nitrate to yield 1% (w/w) silver nitrate impregnation on drying; the slurry was applied to glass plates at a thickness of 0.25 mm, air dried, and the plates were stored in the dark. These plates were used in studies with unlabeled heptachlor, isodrin, thiodan isomers, lindane, DDT, and DDD. After development with hexane-ethyl acetate (99:1), the plates were exposed to ultraviolet light to give darkened areas corresponding to the location of organochlorine compounds (Bontoyan, 1966).

Gas–liquid chromatography (glc) for analysis of the chlorinated hydrocarbon insecticide chemicals was accomplished using a Microtek 220 chromatograph equipped with an all-glass system and a nickel electron capture detector. The column was 3% (w/w) QF-1 on hexamethyldisilizane-treated Chromosorb W, 80–100 mesh. The injector, column, and detector temperatures were 230°, 200°, and 210° C, respectively. Retention times, in minutes, were: aldrin—7.2, photoaldrin—29.0, dieldrin—19.0, photodieldrin—82.2, isodrin—9.2, photoisodrin—28.1, endrin—25.3, endrin aldehyde—50.9, endrin ketone—79.2, heptachlor—6.1, photoheptachlor—10.4, low-melting thiodan isomer—19.5, high-melting thiodan isomer—34.0, lindane—6.0, DDT—27.9, and DDD—26.0.

Photoalteration on Plant Surfaces. The primary leaves (weight approximately 300 mg and dorsal surface area of about 20 cm²) of young bean plants (Pinto variety, grown in vermiculite) were treated with the potential photosensitizer in 50 μl of methanol by spreading the solution evenly over the dorsal surface, with the aid of a microsyringe, to give photosensitizer levels up to 100 ppm based on the fresh leaf weight. After the solvent had evaporated, normally 1 to 2 min, the leaves were treated in a like manner with the labeled pesticide chemical (3 μg in 50 μl of methanol) giving a 10 ppm deposit. The two primary leaves of each plant were used as replicates, and two to four replicates were made. Immediately after application of the radio-labeled pesticide chemical, the plants were placed in direct sunlight, normally for 1 hr. The radioactivity remaining on the leaf surface after this time was recovered by cutting the leaf at the petiole and soaking for 3 min in a beaker containing 10 ml of ether and a small amount of sodium sulfate, followed by a shorter 5 ml ether rinse. The extracts were quantitated for radiocarbon content by scintillation counting of aliquots, then concentrated, spotted for tlc, and the photodecomposition products resolved and quantitated as previously described.

Photoalteration on Glass Surfaces. Certain pesticide chemical–photosensitizer interactions were examined utilizing deposits on glass surfaces by applying the appropriate quantity of photosensitizer (in 10 μl of methanol) uniformly over a glass cover slip of 18 mm diameter. After solvent evaporation, the labeled pesticide was applied, also in 10 μl of methanol, and the treated glass was exposed to sunlight for 1 hr. Each experiment was replicated two or more times. After exposure, radiocarbon remaining on the glass surface was removed by rinsing with ether, and the ether rinses were quantitated and subjected to tlc analysis.

Photoalteration in the Presence of Chloroplasts. Spinach chloroplasts isolated in sorbitol medium from greenhouse-grown spinach leaves as described by Kalberer *et al.* (1967) were supplied by B. B. Buchanan (University of California, Berkeley). The chloroplasts were sedimented from the isolation medium by centrifugation, washed three times with distilled water by resuspension and recentrifugation, and then suspended in distilled water to give 100 μg equivalent of chlorophyll (estimated on acetone extracts according to Arnon, 1949) per ml of suspension. The labeled pesticide (3 μg in 50 μl of methanol) was added to the chloroplast suspension (1 ml) in a 5-ml beaker and the beakers were placed in sunlight for either 15 min or 1 hr in a holder such that the beakers were tilted directly toward the sun. Two types of controls with single factor variations were used: the first involved no exposure to sunlight to determine if light was the limiting factor; the second involved deletion of the chloroplasts to determine if chloroplasts were the limiting factor. Each experiment was replicated twice. After exposure, the labeled compounds were extracted from the aqueous phase by partitioning four times with ether, followed by quantitation of the radiocarbon content of both the organic and aqueous phases. The ether extract was then dried with sodium sulfate and subjected to tlc analysis and radioautography. Radioactive products remaining in the aqueous phase on extraction were considered to be decomposition products.

Identification of Photodecomposition Products. Aldrin-C^{14}, dieldrin-C^{14}, and endrin-C^{14} were exposed to sunlight on bean leaves, with or without photosensitizers. The ether rinses from these leaves were fortified with unlabeled authentic photoisomer(s) for tlc cochromatography studies using various combinations of eight different solvent systems for two-dimensional development resulting in R_f values of 0.05 to 0.95 for the compounds examined. The systems used for aldrin and dieldrin and their degradation products were: carbon tetrachloride-methylene chloride (4:1), hexane, hexane-acetone (8:1), hexane-acetone-methanol (16:1:1), hexane-benzene (4:1), hexane-ether (4:1), methylene chloride, and pentane-methylene chloride (4:1). With the endrin isomers and analogs, the solvents were: benzene-hexane (3:1), carbon tetrachloride-methylene chloride (4:1), ether-hexane (4:1), hexane-acetone (8:1), hexane-acetone-methanol (16:1:1), hexane-chloroform (1:1), hexane-ethyl acetate (4:1), and pentane-methylene chloride (1:1). Radioactive compounds were detected by radioautography and unlabeled compounds by spraying the plates lightly with diphenylamine in acetone (5% w/v) (a modification from Faucheux, 1965) and developing the spots under strong ultraviolet light (General Electric germicidal lamp G15T8, 15 W) for 15 min. Coincidental size, shape, and position of the darkened areas on the film due to the radioactive unknowns and the dark spots on the tlc plates due to the authentic unlabeled compounds in each of the eight solvent systems were considered to constitute tentative product identification.

Glc comparisons of known and unknown compounds were made on the basis of retention times obtained by separate, alternate injections within a few minutes time span of the authentic photoisomers and the unknowns suspected to be identical to the authentic photoisomers. Identical retention times were considered to constitute cochromatography. With isodrin and heptachlor, for which labeled compounds were not available, identification of photoisomers was based primarily on glc studies comparing the unknown compounds formed on bean leaves with the authentic photoisomers. However, limited tlc investigations were conducted in support of the glc findings.

Table I. Activity of Triplet-State Photosensitizers and Pesticide Chemicals in Accelerating the Photoalteration of 10 ppm Deposits of Dieldrin-C^{14} on Growing Bean Leaves Exposed to Sunlight for 1 Hr

Sensitizers[a]	Recovery of Applied Radiocarbon as Photoalteration Products (%) at Indicated Sensitizer Level (ppm)	
	10	100
No photosensitizer	... 4.5 $\pm$ 0.5 ...	
Abate	3.1 $\pm$ 0.3	15.3 $\pm$ 1.3
Anthraquinone	5.1 $\pm$ 1.0	3.5 $\pm$ 0.2
Benzil	4.4 $\pm$ 0.3	4.5 $\pm$ 0.2
Benzophenone	6.5 $\pm$ 0.6	19.4 $\pm$ 0.6
4,4'-Bis(dimethylamino)-benzophenone	18.6 $\pm$ 0.2	11.8 $\pm$ 0.6
Carbazole	6.0 $\pm$ 0.6	7.0 $\pm$ 1.3
Dibenzothiophene	9.3 $\pm$ 1.0	22.4 $\pm$ 1.1
4,4'-Dichlorobenzo-phenone	13.4 $\pm$ 2.8	18.0 $\pm$ 2.6
Flavone	20.2 $\pm$ 2.3	33.4 $\pm$ 1.5
Rotenone	47.0 $\pm$ 1.4	73.9 $\pm$ 2.8
Tetradifon	11.5 $\pm$ 1.1	53.0 $\pm$ 3.6
Thioxanthone	23.8 $\pm$ 7.5	22.7 $\pm$ 0.2
Triphenylamine	19.6 $\pm$ 1.6	59.6 $\pm$ 1.0
Xanthone	33.7 $\pm$ 1.0	48.2 $\pm$ 7.7

[a] Compounds showing moderate to high photosensitizing activity against cyclodienes when tested as deposits on silica gel chromatoplates (Ivie and Casida, 1971).

For identification of DDT photoproducts produced by triphenylamine sensitization, DDT-C^{14} was exposed to sunlight on bean leaves in the presence of triphenylamine. The leaves were then rinsed in ether and these rinses were fortified with unlabeled DDT analogs suspected to be photodecomposition products. After spotting for tlc, the plates were developed two-dimensionally using combinations of the following eight different solvent systems: cyclohexane, cyclohexane-ether (8:1), heptane-chloroform (6:1), heptane-ethyl acetate (8:1), hexane, hexane-benzene (4:1), pentane, methylene chloride (10:1), and 2,2,4-trimethylpentane. Unlabeled standards were detected by viewing the plates under ultraviolet light and radioactive compounds were detected by radioautography. Identical tlc behavior of radioactive DDT photodecomposition products and unlabeled standards in each of the eight solvent systems studied constituted tentative product identification.

RESULTS

Photosensitized Alteration of Dieldrin-C^{14} on Bean Foliage. When the compounds showing moderate to high activity as photosensitizers against cyclodienes on silica gel chromatoplates (Ivie and Casida, 1971) were screened against dieldrin-C^{14} on bean leaves, rotenone was the most effective material in accelerating the photoalteration of the labeled dieldrin (Table I). At 10 ppm, rotenone converted approximately half of the applied dieldrin to a major photoalteration product, which was identified by tlc and glc as photodieldrin. Other sensitizers which were active against dieldrin also acted almost exclusively in converting dieldrin to its photoisomer, with the exception of triphenylamine, which resulted in the formation of several photoproducts in addition to photodieldrin. The sensitizing properties of triphenylamine against dieldrin and other chlorinated hydrocarbons have been attributed to photodechlorination involving charge transfer complexes (Ivie and Casida, 1971; Miller and Narang, 1970). 4,4'-Bis(dimethylamino)benzophenone and thioxanthone, although quite active against dieldrin at 10 ppm, did not produce greater photoisomerization when tested at the higher con-

Table II. Photosensitizing Activity of Rotenone on 22 Radio-Labeled Pesticide Chemicals Exposed to Sunlight on Bean Leaves for 1 Hr

C[14]-Pesticide Chemical (10 ppm)[a]	Recovery of Applied Radiocarbon as Photodecomposition Products (%) at Indicated Rotenone Level (ppm)		
	0	10	100
Aldrin	5.3 ± 0.7	17.6 ± 3.1	50.2 ± 0.4
Dieldrin	4.5 ± 0.5	47.0 ± 1.4	73.9 ± 2.8
Endrin	1.8 ± 0.2	15.4 ± 1.6	42.5 ± 5.6
DDT	4.0 ± 0.2	7.9 ± 0.2	17.4 ± 0.6
Dyfonate	10.9 ± 1.8	11.8 ± 0.6	31.7 ± 2.5
Malathion	8.6 ± 0.4	8.2 ± 0.2	13.6 ± 0.2
Sumithion	5.4 ± 0.8	10.7 ± 0.4	38.6 ± 2.5
Baygon	0.8 ± 0.2	1.0 ± 0.2	3.7 ± 0.2
Carbaryl	2.8 ± 0.2	4.0 ± 0.2	8.8 ± 0.4
Mesurol	12.6 ± 0.3	13.0 ± 0.7	21.0 ± 5.8
Pirimicarb	3.8 ± 0.3	6.6 ± 1.2	11.3 ± 0.3
SD-8530	5.2 ± 0.2	7.0 ± 0.7	19.8 ± 0.7
Allethrin	37.0 ± 1.3	38.5 ± 0.6	51.1 ± 0.7
Dessin	19.2 ± 0.4	32.8 ± 5.0	70.1 ± 2.9
DNBP	5.6 ± 0.2	9.4 ± 0.4	21.1 ± 1.0
Piperonyl butoxide	12.5 ± 0.2	24.2 ± 2.7	38.0 ± 0.2

[a] Rotenone showed no significant photosensitizing activity against the following C[14]-compounds: diazinon, Imidan, isolan, Zectran, pyrethrin I, and 2,4-D.
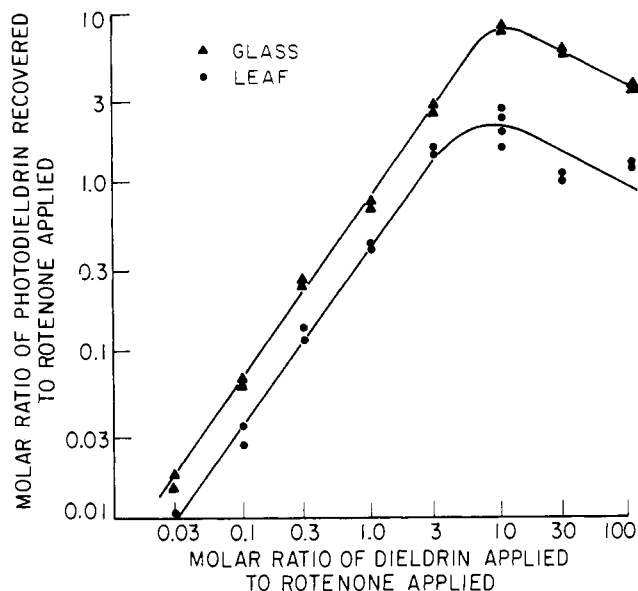
Figure 2. Effect of varying the molar ratio of dieldrin to rotenone on the extent of photodieldrin formation on both glass and leaf surfaces exposed to sunlight for 1 hr. Rotenone levels were constant at 0.6 μg per glass cover slip and 3.0 μg per bean leaf, while dieldrin concentrations were varied

Table III. Photosensitizing Activity of Rotenoids and Related Compounds in the Photoconversion of Dieldrin-C[14] to Photodieldrin-C[14] on Bean Leaves Exposed to Sunlight

Compounds[a]	Relative Potency (Mole Equivalent, Rotenone = 100)
4-Chromanone Moiety Present	
Rotenone and Derivatives	
Rotenone; 6',7'-dihydrorotenone; isorotenone; rotenolone I; 6aβ,12aβ-dihydro-6H-rotoxen-12-one; 4',5'-dihydrodeguelin; neotenone	100
8'-Hydroxyrotenone; epirotenone	40–50
Deguelin; 6aβ,12aβ-dihydro-2,3-dimethoxy-9-hydroxy-8-(3-methylbutyl)-6H-rotoxen-12-one	20–30
Sumatrol	5–10
Others	
Flavanone (2-phenylchromanone)	100
2,2-Dimethyl-7-hydroxy-4-chromanone; 2,2-dimethyl-7-acetyloxy-4-chromanone	30–40
4-Chromanone	<1
4-Chromanone Moiety Absent	
Rotenone Derivatives	
Spiro rotenone isomer	70–80
12,12a-Rotenone diol; rotenone oxime	10–15
6a,12a-Dehydrorotenone; 6a,12a-dehydro-6',7'-dihydrorotenone; 6a,12a-dehydrodeguelin; rotenone enol acetate; rotenonone; derrisic acid; derritol	<1
Others	
Xanthone	20–30
Flavone (2-phenylchromone); thioxanthone	10–15
Anthrone	1
2-Acetyl-1-tetralone; fluoren-9-one; 4-propionyloxy-3-chromanone	<1

[a] For nomenclature and structures of the rotenone derivatives, see Crombie (1963).

centration. Tetradifon, triphenylamine, and xanthone were on the order of tenfold less active than rotenone, whereas anthraquinone, benzil, and carbazole were essentially inactive under the conditions of this study.

Photosensitization of dieldrin-C[14] conversion to photodieldrin-C[14] results in an increase of total residues remaining on the leaves, since photodieldrin is more persistent on the plant surface than is the parent cyclodiene. The persistence of photodieldrin is apparently due to its relatively low volatility in comparison to dieldrin. Labeled dieldrin is rapidly lost from the leaf surface in the absence of an effective photosensitizer (approximately 70% loss in 1 hr), and little of the radiocarbon is found in the leaf residue, as indicated by combustion of the extracted tissue and radioassay by the procedure of Krishna and Casida (1966).

Photosensitizing Activity of Rotenone Against Dieldrin-C[14] on Leaf and Glass Surfaces. Similar patterns of dieldrin sensitization are observed when various dieldrin levels are added to rotenone deposits of 3.0 μg per leaf and 0.6 μg per glass cover slip, followed by exposure to sunlight (Figure 2). The efficiency of rotenone in catalyzing dieldrin photoconversion in these studies is greater on glass than on bean leaves; one molecule of rotenone acting under optimal conditions results in the conversion of more than five dieldrin molecules to photodieldrin. A visible surface film results from high dieldrin deposits, and the possibility of reduced efficiency of light absorption may account for the apparent inhibition of the sensitizing action of rotenone at the higher dieldrin levels studied.

Effects of Rotenone on Other Pesticides. The activity of rotenone as a photosensitizer on foliage is apparently higher against cyclodienes capable of undergoing photoisomerization than with other compounds, as determined by tests involving rotenone combined individually with each of 22 radio-labeled pesticide chemicals (Table II). Rotenone is quite effective in enhancing the rate of aldrin-C[14] photoisomerization to photoaldrin on bean leaves, but some photodieldrin is also produced. Endrin-C[14], in the presence of rotenone and

Interval of Rotenone Photodecomposition (Hr) before Dieldrin Applied	Residues of Rotenone-Derived Products at Time of Dieldrin Application (% of Total Applied)[a]			Recovery of Applied Dieldrin-C^{14} as Photo-dieldrin-C^{14} 1 Hr After Dieldrin Application (%)[b]
	Rotenone	Rotenolone I	Other	
0	89.9 ± 1.1	3.6 ± 0.2	4.4 ± 0.5	47.7 ± 3.0
0.25	58.2 ± 3.7	13.2 ± 0.8	19.3 ± 0.4	43.3 ± 2.5
0.5	37.4 ± 8.7	16.9 ± 1.0	32.7 ± 4.2	36.0 ± 4.2
1.0	22.1 ± 4.0	16.4 ± 0.3	39.3 ± 2.7	24.5 ± 4.0
2.0	8.4 ± 0.1	11.5 ± 1.6	38.2 ± 1.6	15.9 ± 0.4
4.0	3.2 ± 0.1	5.4 ± 0.5	30.6 ± 1.1	14.4 ± 1.5
8.0	3.2 ± 0.2	4.8 ± 0.2	28.3 ± 4.1	10.9 ± 1.2
No rotenone	...	...	...	3.4 ± 0.2

[a] Determined by application of rotenone-C^{14} (10 ppm) and tlc analysis at stated intervals. [b] Leaves receiving 10 ppm dieldrin-C^{14} were pretreated at the appropriate interval with 10 ppm unlabeled rotenone.

sunlight, isomerizes to form the ketone and aldehyde isomers as major products, but several other derivatives are formed as well. None of these products, however, cochromatograph with either the alcoholic endrin isomer or the acid produced by oxidation of the isomeric aldehyde (Phillips et al., 1962). While 13 of the 19 compounds other than cyclodienes can be photosensitized by rotenone, sensitization requires a high rotenone level in most cases. The identity of the products resulting from sensitization of these other pesticide chemicals was not investigated. Some care must be taken in interpreting the data in Table II, since the application of large amounts of rotenone reduced the volatility (or less likely, penetration) of some but not all of the test compounds, which may have allowed photodecomposition products to accumulate. It seems likely, however, that the primary effect of rotenone against the compounds studied is through action as a photosensitizer.

When tested against several unlabeled chlorinated hydrocarbons, rotenone was effective in sensitizing isodrin and heptachlor conversion to products which behave identically on glc and tlc with their authentic photoisomers (Figure 1). Unlabeled DDT, DDD, lindane, and thiodan are affected to a much less degree or not at all by rotenone.

Activity of 2- or 3-Substituted-4-chromanones in Photosensitizing Dieldrin Isomerization. The finding that rotenone is a potent sensitizer for dieldrin photoisomerization on bean leaves prompted studies of rotenone analogs and related compounds to determine the minimal structural requirement for high sensitizing activity. These experiments involved application of the test compound at 10 or 100 ppm and of dieldrin-C^{14} at 10 ppm to bean leaves and determination, on a molar basis, of the amount of rotenone required in the same experiment to duplicate the degree of photodieldrin formation on exposure to sunlight for 1 hr.

Table III indicates that high photosensitizer activity resides in compounds with the 2- or 3-substituted-4-chromanone moiety. Each of the 15 compounds tested which contains this group shows considerable photosensitizing activity, although the degree of activity varies with the other substituents present. For example, sumatrol (11-hydroxyrotenone) is 10–20 times less active than rotenone, possibly due to chelation of the hydroxyl and carbonyl groups. Other activity differences among the 2- or 3-substituted-4-chromanones may relate to differences in volatility, penetration, or photostability on the leaf surface, in addition to direct substituent effects on photosensitizing activity. Apparently, substituents at positions 6, 7, and 8 on the 4-chromanone moiety do not greatly affect the photosensitizing activity. The 4-chroma-

none group is not sufficient in itself, because the unsubstituted compound is essentially inactive as a photosensitizer.

Most of the rotenone derivatives which do not contain the 4-chromanone moiety show no photosensitizing activity, but there are three apparent exceptions: spiro rotenone isomer, rotenone oxime, and 12,12a-rotenone diol. The spiro isomer and rotenone oxime may contain small amounts of rotenone before or after exposure to sunlight, and 12,12a-rotenone diol may owe its activity to rotenolone on the same basis. However, the spiro compound quite possibly is active in itself, since constriction of the rotenone C-ring to form a substituted coumaranone derivative may not be a critical alteration. Unsaturation in the 6a,12a-position of the rotenoids, giving the dehydrorotenone analogs, results in essentially complete activity loss. However, some 4-chromone compounds, which are 2,3-unsaturated chromanones, have moderate photosensitizing activity, i.e., flavone (2-phenylchromone), xanthone, and the related thioxanthone.

Effects of Rotenone Photodegradation on Sensitizing Activity. Rotenone undergoes rapid photodecomposition on plant foliage, resulting in loss of ability to catalyze the photoconversion of dieldrin to photodieldrin (Table IV). The half-life of rotenone under the conditions studied is less than 30 min, and photodecomposition of the surface residue is essentially complete within 4 hr. However, rotenone is converted in significant amounts to rotenolone I, which is as active as the parent compound in sensitizing dieldrin photoisomerization (Table III). As Table IV indicates, the decline in the amount of rotenone and rotenolone I present on the leaves at the time of dieldrin application is paralleled by a reduction in the amount of photodieldrin recovered after 1 hr additional sunlight exposure. Although much of the reduction in sensitizer activity is due to loss of the rotenone deposit by volatilization or penetration, it is probable that the persisting residue, other than rotenone and rotenolone, has little or no photosensitizing activity. This is because rotenone photodegradation, at least in solutions exposed to artificial light sources, involves not only formation of rotenolone I but also rapid disruption of the 4-chromanone moiety (Cheng and Casida, 1970).

Rotenone Sensitization of Dieldrin Isomerization Following Application of Commercial Formulations to Bean Plants. Water emulsions prepared from commercial emulsifiable concentrate formulations of dieldrin were applied to bean foliage with a hand sprayer. Rotenone incorporated into the formulations or sprayed on the leaves before or after dieldrin application resulted in the production of significant amounts of photodieldrin on 1 hr of exposure to sunlight.

Table V. Sensitizing Effects of Aqueous Suspensions of
Spinach Chloroplasts on the Photoalteration of
23 C¹⁴-Pesticide Chemicals Exposed to Sunlight

C¹⁴-Pesticide Chemical[a]	Photodecomposition (%)	
	No Chloroplasts	Chloroplasts
Dyfonate	13.7 ± 1.1	21.6 ± 0.2
Mesurol	1.7 ± 0.9	17.0 ± 2.4
Zectran	24.2 ± 3.7	68.0 ± 1.2
Allethrin[b]	11.1 ± 0.6	30.9 ± 0.2
Pyrethrin I[b]	23.2 ± 0.4	34.0 ± 0.6
Dessin	28.0 ± 9.8	95.5 ± 0.3

[a] Chloroplasts showed no effects on the following C¹⁴-compounds: aldrin, dieldrin, endrin, DDT, diazinon, Imidan, malathion, Sumithion, Baygon, carbaryl, isolan, pirimicarb, SD-8530, rotenone, DNBP, piperonyl butoxide, and 2,4-D. [b] Fifteen min exposure to sunlight; all others 1 hr exposure.

Similar results were obtained when the plants were dusted with commercial rotenone powders followed by spraying of the dieldrin formulation and exposure to sunlight. Without addition of rotenone no photodieldrin was detectable by the tlc analysis method and chromogenic reagents used. These findings suggest that rotenone should accelerate the photo-isomerization of dieldrin residues in the environment if applied in a way to make contact with the dieldrin residues. As discussed previously, however, rotenone would probably retain its effectiveness as a sensitizer for only a short period in the environment, due to its own rapid photodecomposition.

Relative Activity of Photosensitizers on Silica Gel and Plant Foliage. The uniquely high photosensitizing activity of rotenone and related substituted-4-chromanones against dieldrin on plant foliage is somewhat surprising, because several other sensitizers are equal to or greater in effectiveness than rotenone when tested on silica gel chromatoplates exposed to sunlight (Ivie and Casida, 1971). Thus, while xanthone is considerably more active than rotenone on silica gel, the opposite relationship occurs on bean leaves. In addition, 4-chromanone is essentially inactive on leaves, but is approximately equal in activity to rotenone when tested on silica gel. These findings suggest that rapid volatilization, penetration, or photodecomposition to inactive products occur when some of these materials are tested on bean leaves but not on silica gel. However, studies of the persistence of these compounds on bean leaves by tlc using ultraviolet light absorption characteristics to monitor the sensitizers indicated that most of the materials were quite persistent on the leaf surface. Therefore, other factors must be considered for the observed activity differences, such as the physical and physicochemical characteristics of the sensitizer in conferring properties appropriate for attraction to or contact with the dieldrin molecule.

Efficiency of Triphenylamine in Photosensitizing DDT Degradation on Bean Foliage. DDT is quite stable on bean leaves but it breaks down rapidly after application of triphenylamine to the plant surface (Figure 3). The activity of triphenylamine as a sensitizer is short-lived, however, as its action is essentially complete within 30 min after application. The products of triphenylamine-sensitized DDT photodecomposition were found by tlc analysis to include DDE and dichlorobenzophenone, but of at least four unidentified products formed, none cochromatographed with DDD. This is somewhat surprising, since Miller and Narang (1970) reported DDD, as well as DDE and dichlorobenzophenone, as photoproducts when DDT and triphenylamine were exposed to artificial light in cyclohexane solution.

Photosensitizing Effects of Spinach Chloroplasts. Aqueous
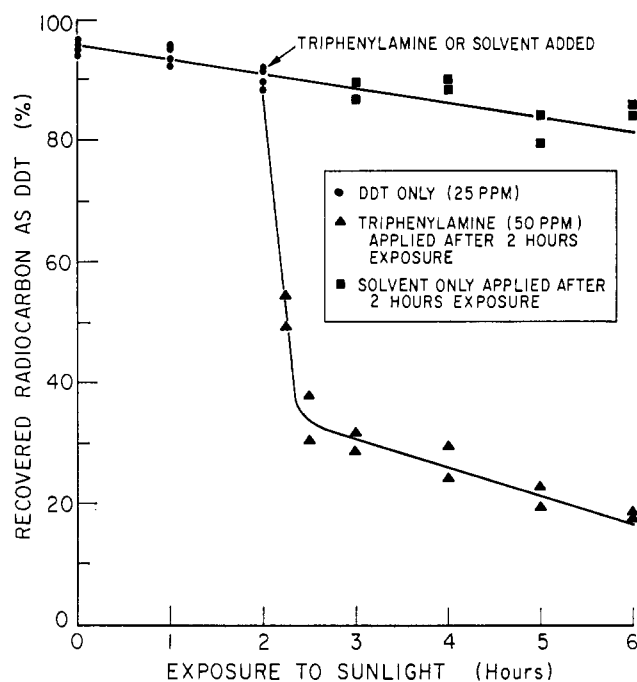

Figure 3. Effect of triphenylamine application on the residual persistence of DDT on bean leaves

suspensions of spinach chloroplasts are effective in accelerating the photodecomposition of six of the 23 C¹⁴-pesticides studied, showing highest activity against the N-methylcarbamates, Mesurol and Zectran, and the dinitrophenol derivative, Dessin (Table V). Chloroplast sensitization is light dependent; the reactions do not proceed in darkness. Of the six compounds affected by chloroplasts, only Dyfonate, pyrethrin I, and Dessin are sensitized to produce significant amounts of products not extractable from the aqueous phase into organic solvent. These water-soluble materials probably result from photodecomposition rather than binding of the parent pesticide to the chloroplasts, because water soluble metabolites were not observed in samples containing chloroplasts but not exposed to sunlight. The finding that chloroplasts show activity against the pyrethroids is not surprising, inasmuch as previous studies have shown that plant pigments in the presence of sunlight accelerate the degradation of crude pyrethrum extract (Brown and Phipers, 1955; Glynne Jones, 1960). The sensitizing activity of chloroplasts is probably due largely or completely to their chlorophyll content, because purified chlorophylls a and b are sensitizers under these conditions, though not as effective as an equivalent amount of chloroplasts.

The extent to which chlorophylls and other natural plant products act as sensitizers for pesticide photodecomposition in the environment is unknown, but these studies suggest that such interactions may be of significance in some cases. In addition to rotenone and the chlorophylls, flavonoid derivatives occurring commonly in some plants may be potential photosensitizers, and FMN has been demonstrated to sensitize the photodecomposition of the substituted urea herbicide, monuron (Sweetser, 1963), and the chloroanilino moieties of several other herbicides (Rosen et al., 1970). Further studies may reveal other naturally occurring sensitizers as well.

DISCUSSION

In the normal use of pesticide chemicals in plant protection, the specific compound to be used and the formulation are

selected, in part, on the basis of obtaining the desired degree of residual persistence on plant foliage. The present study indicates that photosensitizing chemicals may constitute an additional factor useful in governing or manipulating the persistence of residues. For example, unusual climatic conditions may result in abnormally high residue levels near harvest time, and these might quickly be reduced by photosensitizer application. The photosensitizer may be a pesticide chemical, and therefore of known toxicological properties, or a chemical not known to have pesticidal activity in itself. It is obvious that if a photosensitizer is to be used, it must be selected on the basis of both photosensitizing activity and toxicological characteristics.

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